# Supplementary appendix

**Contents**

## Supplementary Methods

### Recruitment of LB patients

LB patients were included online (www.tekenradar.nl) and through the participating clinical LB centers. Potential participants were recruited in several ways. Nationwide, posters were put up in general practitioners' waiting rooms, popular and social (news) media gave attention to the study at various moments during the study, patient societies were involved in the recruitment, and general practitioners as well as neurologists, dermatologists and rheumatologists were informed through specific field media and meetings. This way, both patients with EM and disseminated LB and their physicians were encouraged to register online for study participation.

### Predefined confounders and permutation tests

To reduce confounding effects as much as possible, i.e. to control for extraneous factors that might be associated with the primary outcome and might differ between the various cohorts, indirect standardization was performed with respect to the LB patients. To this end, strata were formed using predefined confounders (age, sex, educational level and comorbidity). The continuous confounders were coded as categorical variables, by using breaks that were chosen after exploring the crude prevalence of persistent symptoms in the LB patients, tick bite and population cohorts, based on the primary scenario for substitution for missing data. This led to the following categorical variables:

- Age: breaks at age 45 and 65 years.
- Sex: male and female.
- Educational level: eight Dutch educational levels were divided into two categories, low and high educational level, to limit the number of strata. University of applied sciences and academic university education were categorized as high educational level.
- Comorbidity: based on the number of reported comorbidities (as listed by the TiC-P) three categories were included (0, 1 and ≥2 comorbidities).

For analyses on patients with disseminated LB, because of their low numbers only two confounders were used. These were sex and comorbidity (the latter in two categories: 0 and ≥1 comorbidities).

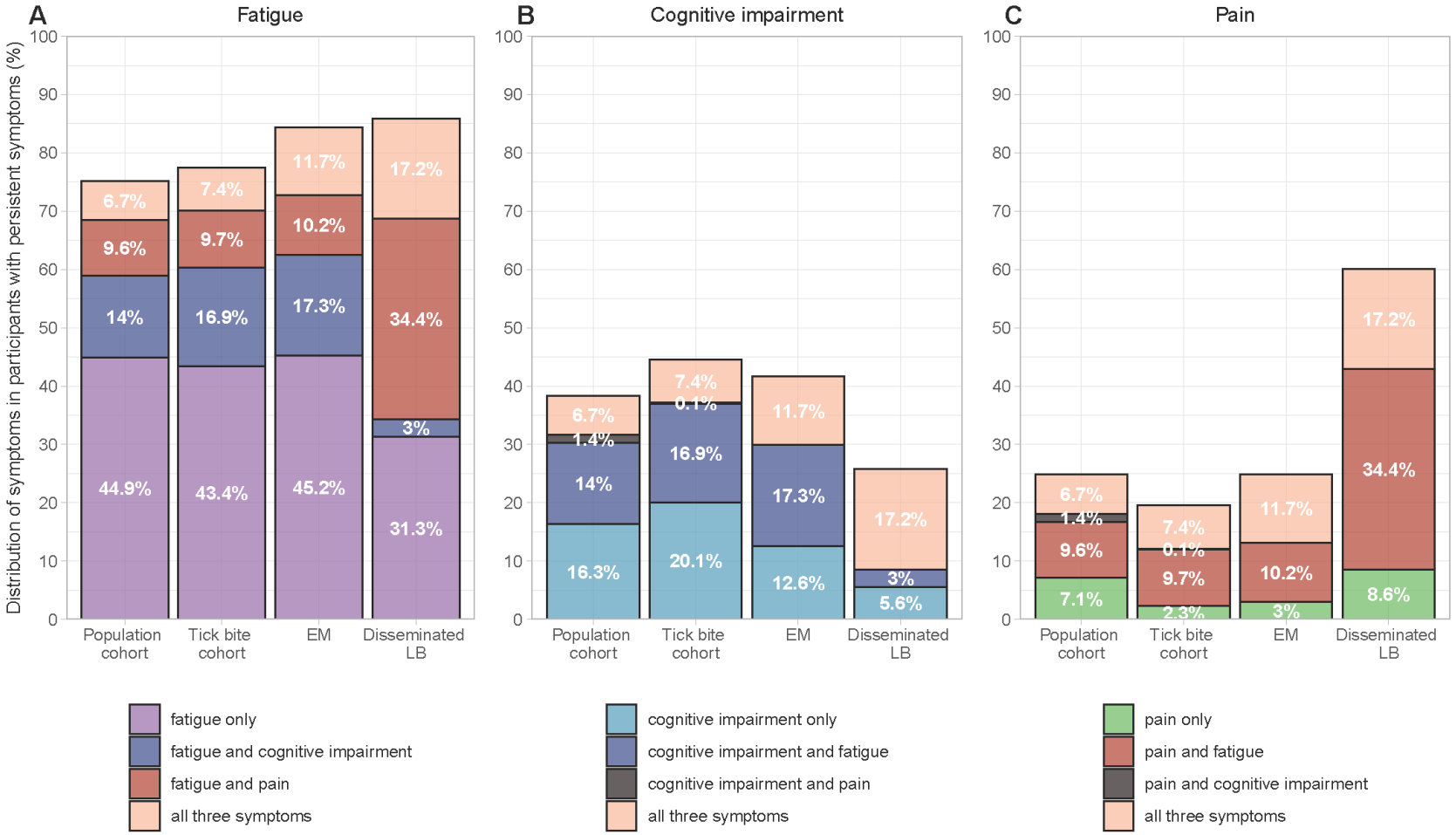
Within each cohort and stratum a sample mean and variance of the mean was determined. These were pooled to an overall mean and variance of the mean, weighted to the relative size of the stratum (mean) and the square of the relative size (variance of the mean) within the LB patients. The 95% confidence intervals were computed through normal approximations from the pooled variances of the mean. For the comparison of the prevalence of persistent symptoms between the LB patients and the reference cohorts, permutation tests based on the sum statistic were used with the same confounders as for the indirect standardisation.[1,2] If strata only contained subjects from one cohort, they were omitted from testing, leading to lower sample sizes.

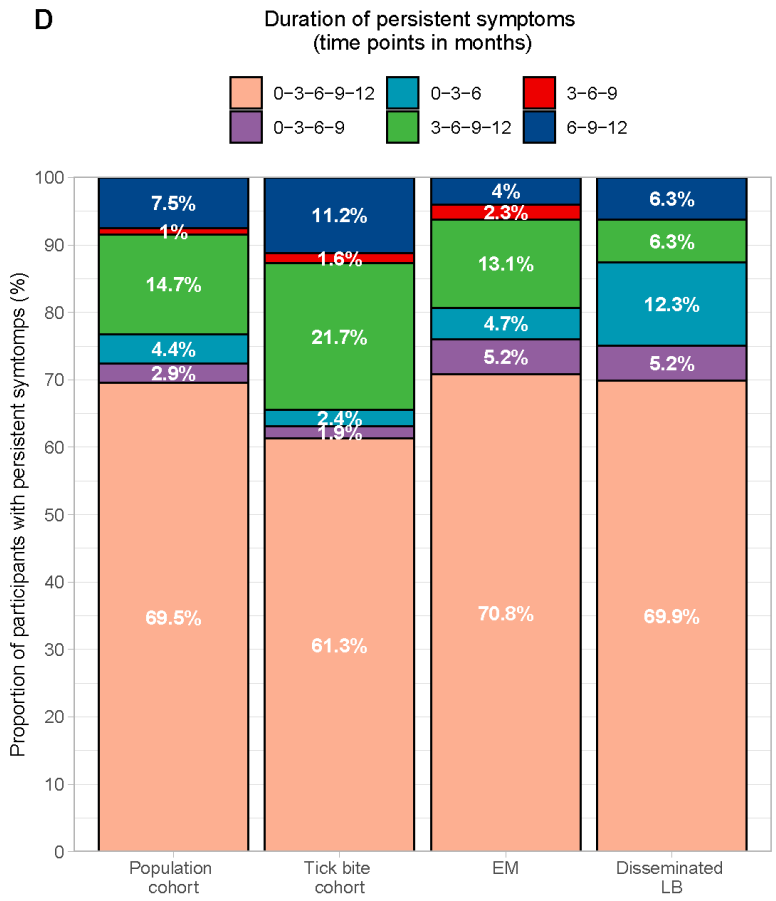
### Linear mixed effects models for assessment of differences in severity over time

Linear mixed effects models were used to construct a standardised symptom severity course and to assess differences in this course between cohorts. The outcome of this model is a function dependent on an intercept, cohort, a quadratic polynomial for time, an interaction between cohort and that polynomial and the used

confounders. A random effect for each individual was used to account for the correlation between observations within an individual. If any p-values for the cohort fixed effects or interactions were significant, the overall course of symptom severity in the assessed cohort was considered to be different from the reference cohort. In the primary analysis, the EM patients and disseminated LB patients were compared with the population and tick bite cohorts. As a secondary outcome analysis, the severity course of chronic symptoms attributed to unconfirmed LB was compared with the severity of symptoms in LB patients meeting the definition for persistent symptoms.

**Supplementary Figure S1: Distribution and duration of symptoms in participants meeting the definition of persistent symptoms**

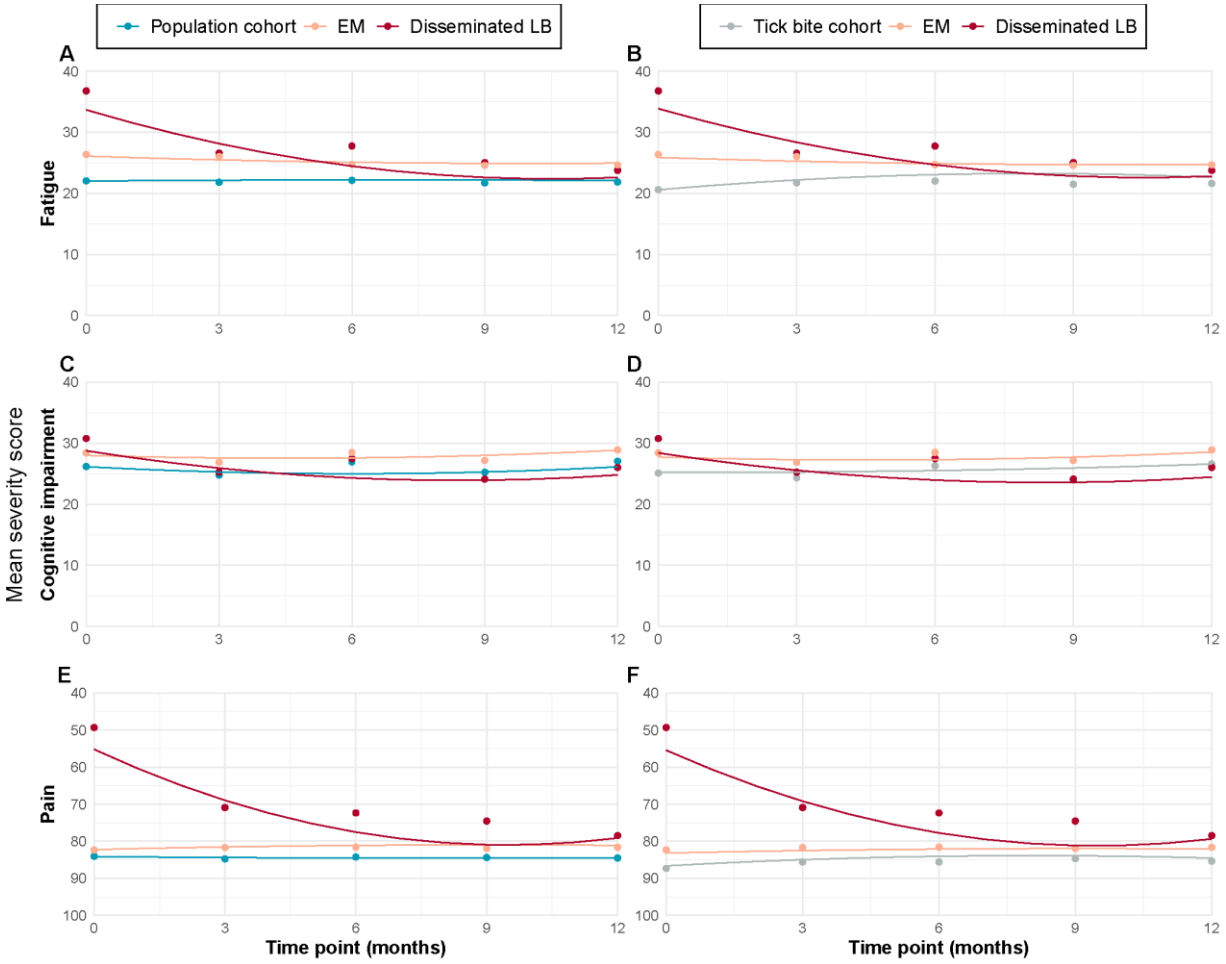





Only the subgroup of participants meeting the definition of persistent symptoms in each cohort are included in this figure. (A-C) The standardised percentage of these participants that report each individual symptom, either or not accompanied by one or both other symptom(s). (D) The duration and time points the persistent symptoms were present in each cohort.

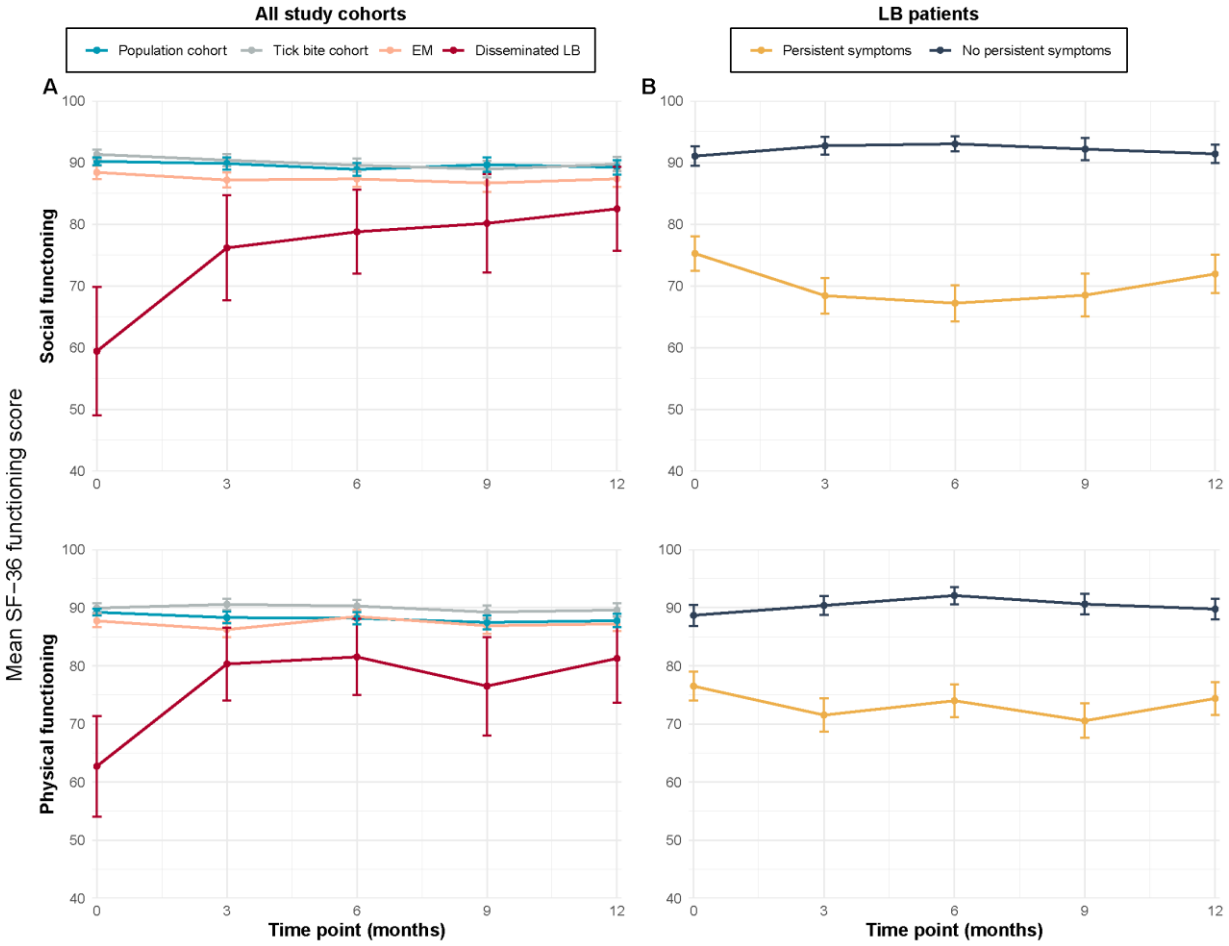
*Abbreviations: EM = erythema migrans, LB = Lyme borreliosis.*

**Supplementary Figure S2: Linear mixed effects models for assessment of differences in severity over time between LB patients and the population and tick bite cohorts**




Linear mixed effects models over the 12 months study period comparing severity over time of fatigue (CIS, subscale fatigue; A and B), cognitive impairment (CFQ; C and D) and pain (SF-36, subscale bodily pain; E and F) in patients with EM or disseminated LB to the population and tick bite cohorts. P-values are provided in Table S8. *Abbreviations: CFQ = Cognitive Failure Questionnaire, CIS = Checklist Individual Strength, EM = erythema migrans, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

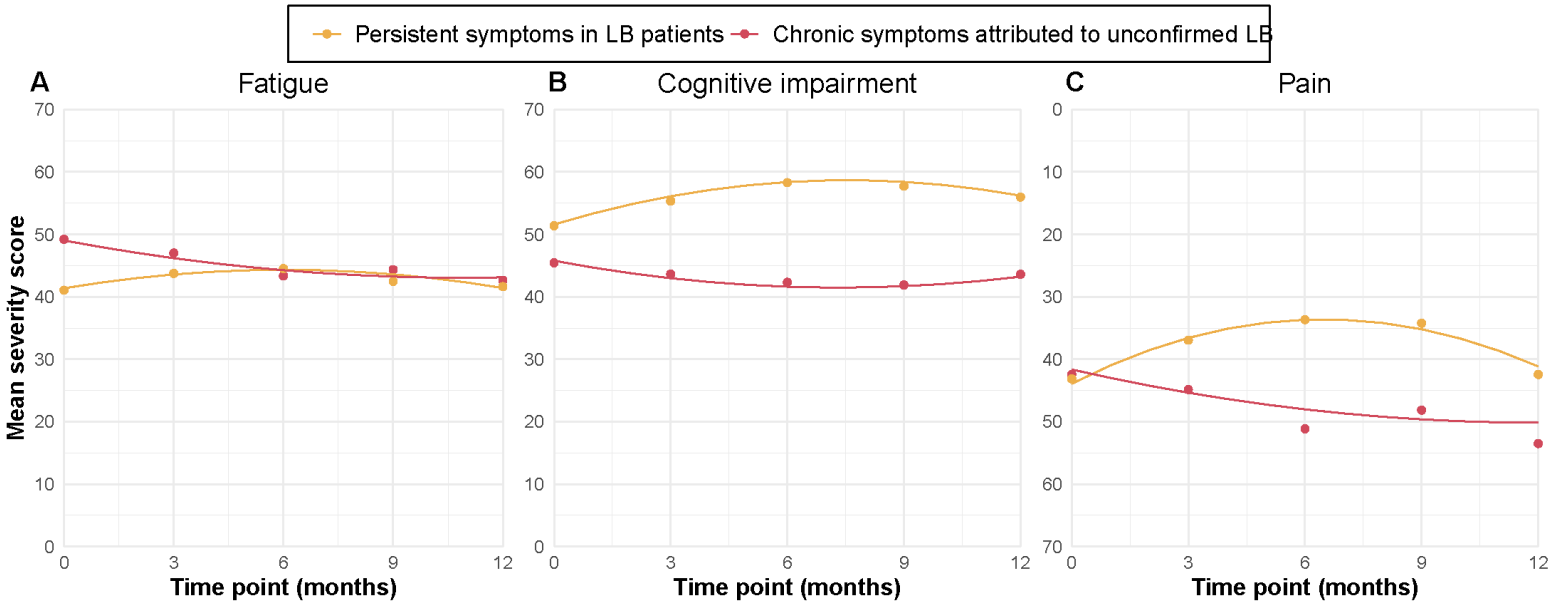
**Supplementary Figure S3: Social and physical functioning in all cohorts and in LB patients with and without persistent symptoms**




Results depict standardised mean scores with 95% confidence intervals on the SF-36 subscales physical and social functioning at the five time points (A) in all cohorts and (B) in LB patients meeting the definition of persistent symptoms and those who did not (based on the primary scenario for substitution of missing data). Lower scores indicate limitations in physical and social functioning.

*Abbreviations: EM = erythema migrans, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

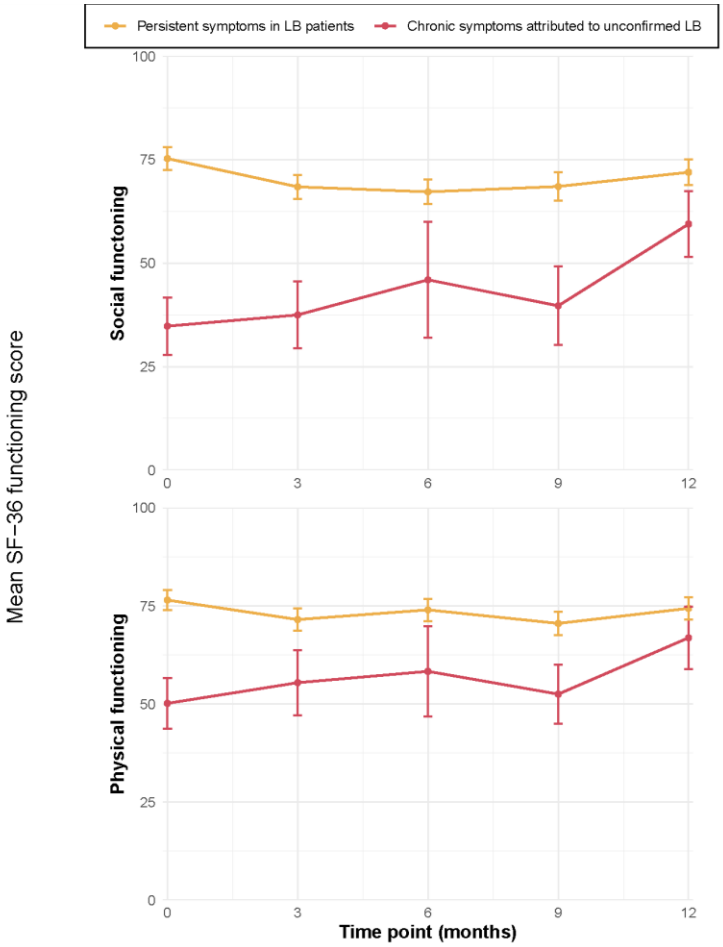
**Supplementary Figure S4: Linear mixed effects models for assessment of differences in severity over time for LB patients meeting the definition of persistent symptoms compared with patients with chronic symptoms attributed to unconfirmed LB**




Linear mixed effects models over the 12 months study comparing severity over time of that fatigue (CIS, A), cognitive impairment (CFQ, B) and pain (SF-36, C) in LB patients meeting the definition of persistent symptoms with patients with chronic symptoms attributed to unconfirmed LB. P-values are provided in Table S8.

*Abbreviations: CFQ = Cognitive Failure Questionnaire, CIS = Checklist Individual Strength, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

**Supplementary Figure S5: Social and physical functioning in LB patients meeting the definition of persistent symptoms and in patients with chronic symptoms attributed to unconfirmed LB**




Results depict standardised mean scores with 95% confidence intervals on the SF-36 subscales physical and social functioning at the five time points in patients with chronic symptoms attributed to unconfirmed LB and in LB patients meeting the definition of persistent symptoms, using the primary scenario for substitution of missing data. Lower scores indicate limitations in physical and social functioning.

*Abbreviations: LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

## Supplementary Table S1: Inclusion and exclusion criteria for the cohort of LB patients

| **Inclusion criteria** | | |
|---|---|---|
| 1. Patients ≥18 years with confirmed proven or probable early localised or disseminated LB manifestation as specified below; | | |
| 2. In case of an EM reported online (at www.tekenradar.nl), the EM has been present <3 months and the clinical diagnosis has been confirmed to the research staff by the general practitioner (criteria for clinical diagnosis are described below); | | |
| 3. Subjects live or stay on the mainland of the Netherlands. | | |
| | | |
| **Clinical and laboratory criteria for confirmed LB** | **Clinical criteria** | **Laboratory criteria** |
| Typical erythema migrans¶ | Centrifugal expanding red or red-bluish macule or ring >5 cm, without vesicles, papulae, desquamation or infiltration, regardless the observation of a tick bite. | None |
| Atypical erythema migrans with observed tick bite¶ | Centrifugal expanding red or bluish-red macule or ring >5 cm, with vesicles, papulae, desquamation or infiltration. | None |
| Atypical erythema migrans without observed tick bite¶ | Centrifugal expanding red or bluish-red macule or ring >5 cm, with vesicles, papulae, desquamation or infiltration. | 1. Serological profile matching early infection*<br>*and/or*<br>2. Positive *B. burgdorferi* s.l. PCR on skin biopsy<br>*and/or*<br>3. Positive *B. burgdorferi* s.l. culture on skin biopsy |
| Early (sub)acute symptoms without EM | Fever (>38.3℃) or subfebrile temperature (37.8-38.3℃) AND myalgia or arthralgia. Symptoms are existing <3 months, and started within 1 month after a documented tick bite. | Serological profile matching early infection* |
| Proven *Borrelial* lymphocytoma | Painless smooth bluish-red nodule or plaque with a diameter of at least 1 cm, usually found on the ear lobe or helix, nipple or scrotum. | 1. Positive *B. burgdorferi* s.l. PCR or culture on skin biopsy and/or positive *B. burgdorferi* s.l. PCR or culture on blood if performed in a participating laboratory<br>*and/or*<br>2. Histopathology showing polyclonal B-lymphocytes infiltration, with positive spirochete staining<br>*and/or*<br>3. Histopathology showing polyclonal B-lymphocytes infiltration, without positive spirochete staining, but with a serological profile matching early infection* |
| Probable *Borrelial* lymphocytoma | Painless smooth bluish-red nodule or plaque with a diameter of at least 1 cm, usually found on the ear lobe or helix, nipple or scrotum. | 1. Histopathology showing polyclonal B-lymphocytes infiltration, without positive spirochete staining<br>*or*<br>2. A serological profile matching early infection* |
| Proven multipele erythema migrans | Multipele red or blue-red skin lesions, oval or round shaped. | 1. Positive *B. burgdorferi* s.l. PCR of culture on skin biopsy<br>*and/or*<br>2. Positive *B. burgdorferi* s.l. PCR of culture on blood (if performed in a participating laboratory) |
| Probable multipele erythema migrans | Multipele red or blue-red skin lesions, oval or round shaped. The lesions are homogeneous and have a sharp border. | A serological profile matching early or late infection* |
| Proven Lyme neuroborreliosis | Meningo-(poly)radiculoneuritis, meningitis, myelitis, encephalitis, cerebral vasculitis (presenting as a cerebrovascular accident), unilateral or bilateral facial palsy, or involvement of other cranial nerves. | 1. Pleiocytosis and intrathecal specific *B. burgdorferi* s.l. antibody formation<br>*and/or*<br>2. Pleiocytosis and elevated CXCL13 in cerebrospinal fluid (CSF)[#]<br>*and/or*<br>3. Positive culture or PCR for *B. burgdorferi* s.l. on CSF |

| | | |
|---|---|---|
| Probable Lyme neuroborreliosis | Meningo-(poly)radiculoneuritis, meningitis, myelitis, encephalitis, cerebral vasculitis (presenting as a CVA), unilateral or bilateral facial palsy, or involvement of other cranial nerves. | 1. Pleiocytosis and a serological profile matching early infection*<br>*or*<br>2. Intrathecal specific *B. burgdorferi s.l.* antibody formation and a serological profile matching early or late infection*<br>*or*<br>3. Elevated CXCL13 in CSF[#] and a serological profile matching early or late infection* |
| Probable Lyme polyneuropathy | Objective polyneuropathy[†] together with skin lesions compatible with an acrodermatitis chronic atrophicans (see criteria ACA). | A serological profile matching late infection* |
| Proven Lyme arthritis | Persistent or recurrent swelling of one or more joints (synovitis), mostly the knee. | A positive PCR or culture of *B. burgdorferi* s.l. on synovial fluid or synovium |
| Probable Lyme arthritis | Persistent or recurrent swelling of one or more joints (synovitis), mostly the knee. | 1. A serological profile matching late infection*<br>*and*<br>2. another causative explanation for the arthritis has been excluded after consultation by a rheumatologist[$] |
| Proven ACA | Red or bluish-red discoloration of the skin with limited swelling end/or atrophy. | A positive PCR or culture of *B. burgdorferi* s.l. on skin biopsy |
| Probable ACA | Red or bluish-red discoloration of the skin with limited swelling end/or atrophy. | A serological profile matching late infection* |
| Probable Lyme carditis | New onset of atrioventricular conduction disorder (first, second or third degree), or new onset of clinical symptoms of a perimyocarditis together with at least one symptom of an early or late disseminated LB <6 weeks before cardiac symptoms started. | 1. A serological profile matching late infection*<br>*and*<br>2. another causative explanation for the carditis has been excluded[‡] |
| Proven Lyme uveitis | Uveitis (anterior, intermedia, posterior, or panuveitis) | A positive PCR *B. burgdorferi* s.l. on vitreous fluid |
| Probable Lyme uveitis | Uveitis (anterior, intermedia, posterior, or panuveitis) | A serological profile matching early infection* |
| | | |
| **Exclusion criteria** | | |
| 1. Subjects unable to provide informed consent or not having sufficient command of the Dutch language; | | |
| 2. Subjects who started antibiotic treatment >4 days before inclusion (for subjects included after online reporting) or >7 days before inclusion (for subjects included through the participating clinical LB centers); | | |
| 3. Subjects who have ongoing signs or symptoms attributed to a previous episode of LB | | |

Alternative causes for symptoms should have been excluded by the primary treating physician. Inclusion criteria were largely based on the European clinical case definitions, as described by Stanek et al.[3]

[¶]In case of EM, physician confirmation was accomplished by a questionnaire, sent to the general practitioners, to check for the clinical criteria and antibiotic treatment. These questionnaires, combined with a photograph of the EM and with clinical information if necessary and available, were used by the researchers to carefully classify EM as 'typical' or 'atypical', for the latter also including the laboratory criteria mentioned in the table. For atypical EM with observed tick bite, the skin lesion had to have occurred at the site of the tick bite. The median duration between tick bite and EM for 147 patients meeting the criteria for atypical EM with observed tick bite was 11 days, and for all but two patients the duration was <60 days (one patient 103 days and one patient 96 days).

*Definition of serological results:

Early infection (symptoms <8 weeks): positive *B. burgdorferi* s.l. IgM EIA/ELISA with positive *B. burgdorferi* s.l. IgM Immunoblot and negative *B. burgdorferi* s.l. IgG Immunoblot, OR positive *B. burgdorferi* s.l. IgM/IgG EIA/ELISA with positive *B. burgdorferi* s.l. IgM Immunoblot and negative *B. burgdorferi* s.l. IgG Immunoblot, AND/OR seroconversion from negative or borderline IgG *B. burgdorferi* s.l. EIA/ELISA to positive IgG *B. burgdorferi* s.l. EIA/ELISA with positive IgG *B. burgdorferi* s.l. Immunoblot, OR seroconversion from negative or borderline IgM/IgG *B. burgdorferi* s.l. EIA/ELISA to positive IgM/IgG *B. burgdorferi* s.l. EIA/ELISA with positive IgG *B. burgdorferi* s.l. Immunoblot.

Late infection (symptoms >8 weeks): positive *B. burgdorferi* s.l. IgG EIA/ELISA with positive *B. burgdorferi* s.l. IgG Immunoblot, AND/OR positive *B. burgdorferi* s.l. IgM/IgG EIA/ELISA with positive *B. burgdorferi* s.l. IgG Immunoblot.

[#]The CXCL-13 cut-off value is laboratory dependent.

[†]As determined by neurological assessment or electromyogram.

[$]Preferably through synovial fluid puncture or synovium biopsy.

[‡]After a cardiologist has been consulted.

*Abbreviations: ACA = acrodermatitis chronic atrophicans, B. burgdorferi s.l. = Borrelia burgdorferi sensu lato, CSF = cerebrospinal fluid, CXCL-13 = C-X-C motif Ligand 13, EIA/ELISA = Enzyme (Linked) Immuno (Sorbent) Assay, EM = erythema migrans, IgM/IgG = Immunoglobulin M or G, LB = Lyme borreliosis, PCR = polymerase chain reaction.*

## Supplementary Table S2: Inclusion and exclusion criteria for the population and tick bite cohorts

| **Inclusion criteria** |
|---|
| 1. Subjects ≥18 years who live or stay on the mainland of the Netherlands; |
| 2. Subjects who have completed the baseline online questionnaire); |
| 3. Subjects who have reported a tick bite without LB manifestation between April 2016 and July 2019 (tick bite cohort) or have been invited for the baseline questionnaire between October 2017 and September 2018 (population cohort). |
| |
| **Exclusion criteria** |
| 1. Subjects who report persistent symptoms (self-)attributed to (possible) previous LB at baseline; |
| 2. Subjects who report use of medication for LB (with exception of prophylaxis) in the previous two weeks (tick bite cohort) or three months (population cohort) at baseline or during follow-up; |
| 3. Subjects who report fever or possible EM after a recent or new tick bite, or a new (possible) LB diagnosis at baseline or during follow-up; |
| 4. Tick bite cohort only: subjects who have reported (possible) LB or fever after a tick bite in the 12 months prior to baseline. |

*Abbreviations: EM = erythema migrans, LB = Lyme borreliosis.*

## Supplementary Table S3: Inclusion and exclusion criteria for the cohort of patients with chronic symptoms attributed to unconfirmed LB

| **Inclusion criteria** |
|---|
| 1. Myalgia, arthralgia, neuralgia, concentration disorders, cognitive impairment, with or without fatigue, present for ≥6 months at baseline; |
| 2. Severity of symptoms assessed by the CIS, CFQ and/or SF-36 questionnaire above the Dutch norm scores; |
| 3. Subjects have a negative serological test for *B. burgdorferi* s.l. (IgG ELISA or C6 IgM/IgG ELISA), but have a history of an unconfirmed suspicion of LB, based on<br>• a positive result for a non-recommended diagnostic test (e.g., cellular tests, CD57 analysis, viable blood analysis, bioresonance), or<br>• onset of disease symptoms that have started within one month after a documented tick bite. |
| |
| **Exclusion criteria** |
| 1. Subjects unable to give informed consent or not having sufficient command of the Dutch language. |

Symptoms were attributed to Lyme borreliosis based on a positive result for one or more non-recommended tests (n=60), e.g. commercially available cellular tests, because of a temporal relationship with a tick bite (n=1), or both (n=4).

*Abbreviations: B. burgdorferi s.l. = Borrelia burgdorferi sensu lato, CFQ = Cognitive Failure Questionnaire, CIS = Checklist Individual Strength, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

## Supplementary Table S4: Sensitivity analyses of the primary outcome

The prevalence of persistent symptoms in all cohorts (primary outcome) was assessed with a primary analysis scenario for the substitution of missing data (Table S4A). To confirm the robustness of the primary outcome, sensitivity analyses were performed with alternative substitution techniques (Table S4B), and with alternative analysis populations (Table S4C).

**Supplementary Table S4A: Primary outcome based on the primary scenario for the substitution of missing data**

| | Reference cohorts | | LB patients | | |
|---|---|---|---|---|---|
| **Scenario** | **Population cohort** | **Tick bite cohort** | **All LB patients** | ***EM*** | ***Disseminated LB**** |
| Primary scenario | 21.2% (19.3-23.1)<br>n=1942 | 23.3% (21.3-25.3)<br>n=1887 | 27.2% (24.7-29.7)<br>n=1084<br>[1] <0.0001<br>[2] 0.016 | 27.2% (24.6-29.8)<br>n=1026<br>[1] 0.00012<br>[2] 0.021 | 34.3% (21.7-46.9)<br>n=58<br>[1] 0.021<br>[2] 0.032 |

**Sensitivity analyses with alternative substitution techniques for missing primary outcome questionnaires**

Sensitivity analyses included three alternative substitution techniques for missing primary outcome questionnaires (Table S4B):

1. No substitution: Only data from participants who had completed sufficient primary outcome observations to be categorized as a case with or without persistent symptoms were included.
2. Interpolate when consistent: Outcome of a missing questionnaire was substituted by the dichotomous outcome (normal or aberrant) of the previous and subsequent questionnaires (only in case outcome of both questionnaires were available and consistent), if available questionnaire scores were not sufficient to be categorized as a case with or without persistent symptoms.
3. Carry backward or forward: Dichotomous (normal or aberrant) outcome of the two subsequent or previous questionnaires were carried backward or forward (provided the outcome of both questionnaires was available and consistent). The mean of the two closest continuous questionnaire scores were used in case the six months' questionnaire was missing only.

**Supplementary Table S4B: Sensitivity analyses with alternative substitution techniques for missing primary outcome questionnaires**

| | Reference cohorts | | LB patients | | |
|---|---|---|---|---|---|
| **Scenario** | **Population cohort** | **Tick bite cohort** | **All LB patients** | ***EM*** | ***Disseminated LB**** |
| No substitution | 15.4% (12.6-18.3)<br>n=706 | 19.2% (15.9-22.5)<br>n=701 | 27.3% (24.0-30.7)<br>n=600<br>[1] <0.0001<br>[2] 0.00091 | 27.5% (24.0-31.0)<br>n=563<br>[1] <0.0001<br>[2] 0.00099 | 28.9% (14.4-43.4)<br>n=37<br>[1] 0.10<br>[2] 0.097 |

| | | | | | |
|---|---|---|---|---|---|
| Interpolate when consistent | 18.3% (15.8-20.9)<br>n=1068 | 21.6% (19.0-24.2)<br>n=1062 | 28.0% (25.1-30.8)<br>n=855<br>[1] <0.0001<br>[2] 0.00073 | 28.2% (25.3-31.1)<br>n=810<br>[1] <0.0001<br>[2] 0.00073 | 33.6% (18.4-48.8)<br>n=45<br>[1] 0,046<br>[2] 0.071 |
| Carry backward or forward | 19.7% (17.7-21.7)<br>n=1649 | 20.5% (18.4-22.6)<br>n=1643 | 25.7% (23.2-28.3)<br>n=991<br>[1] 0.00012<br>[2] 0.0018 | 25.8% (23.1-28.4)<br>n=939<br>[1] 0.00011<br>[2] 0.0022 | 33.6% (19.6-47.6)<br>n=52<br>[1] 0.069<br>[2] 0.025 |

**Sensitivity analyses in alternative analysis populations**

Prevalence of persistent symptoms, according to the primary substitution scenario, was assessed in three alternative analysis populations (Table S4C):

1. Patients with highly probable EM based on one or more of the following criteria: Seroconversion in IgM or IgG blot (n=16), seroreversion in IgM blot (n=44), *B. burgdorferi* s.l. skin culture or PCR positivity (n=22) or probable EM based on uniform photograph assessment by three independent assessors (n=390).
2. Participants without self-reported severe fatigue, concentration disorder or pain lasting during ≥3 months in the year prior to study participation.
3. Participants with ≥3 questionnaires available.

**Supplementary Table S4C: Sensitivity analyses in alternative analysis populations**

| | **Reference cohorts** | | **LB patients** | | |
|---|---|---|---|---|---|
| **Analysis population** | **Population cohort** | **Tick bite cohort** | **All LB patients** | ***EM*** | ***Disseminated LB**** |
| Highly probable EM patients | 21.7% (19.7-23.7)<br>n=1942 | 23.5% (21.5-25.5)<br>n=1887 | NA | 31.1% (27.1-35.1)<br>n=436<br>[1] <0.0001<br>[2] 0.0021 | NA |
| Participants without severe fatigue, concentration disorder, or pain, ≥3 months in the year prior to participation | 16.8% (14.9-18.6)<br>n=1796 | 19.9% (17.9-21.8)<br>n=1802 | 24.4% (21.3-27.4)<br>n=714<br>[1] <0.0001<br>[2] 0.013 | 24.6% (21.5-27.7)<br>n=695<br>[1] <0.0001<br>[2] 0.0087 | 19.9% (0-49.0)<br>n=19<br>[1] 1.00<br>[2] 1.00 |
| Participants with ≥3 questionnaires available | 18.7% (16.6-20.8)<br>n=1435 | 20.5% (18.3-22.6)<br>n=1458 | 26.9% (24.3-29.4)<br>n=1016<br>[1] <0.0001<br>[2] 0.00031 | 26.9% (24.3-29.6)<br>n=962<br>[1] <0.0001<br>[2] 0.00045 | 32.3% (19.7-45.0)<br>n=54<br>[1] 0.013<br>[2] 0.12 |

Tables S4A-C depict prevalence of persistent symptoms (95% CI) and total number of subjects per cohort according to the primary scenario (A), each sensitivity scenario for the substitution of missing data (B), and each alternative analysis population (C). Permutation tests based on sum statistics were used to assess differences in prevalence of persistent symptoms between LB patients (and the groups of EM and disseminated LB patients) and the population ([1]) and tick bite ([2]) cohort.

*Disseminated LB patients were stratified by two out of four confounders (comorbidity and sex).

*Abbreviations: B. burgdorferi s.l. = Borrelia burgdorferi sensu lato, CI = confidence interval, EM = erythema migrans, LB = Lyme borreliosis, PCR = polymerase chain reaction.*

## Supplementary Table S5: Numbers of completed questionnaires at baseline and during follow-up per cohort

| | | Baseline | 3 months | 6 months | 9 months | 12 months |
|---|---|---|---|---|---|---|
| **Primary outcome measures** | | | | | | |
| CIS (subscale fatigue severity)[1] | All LB patients (n=1135) | 1121 | 787 | 967 | 883 | 924 |
| | *EM (n=1076)* | 1067 | 744 | 913 | 832 | 873 |
| | *Disseminated LB (n=59)* | 54 | 43 | 54 | 51 | 51 |
| | Chronic symptoms attributed to unconfirmed LB (n=65) | 59 | 44 | 49 | 41 | 43 |
| | Population cohort (n=4000) | 4000 | 1544 | 1223 | 915 | 1212 |
| | Tick bite cohort (n=2405) | 2405 | 1584 | 1293 | 949 | 1111 |
| CFQ | All LB patients (n=1135) | 1121 | 1017 | 968 | 886 | 924 |
| | *EM (n=1076)* | 1067 | 961 | 914 | 835 | 873 |
| | *Disseminated LB (n=59)* | 54 | 56 | 54 | 51 | 51 |
| | Chronic symptoms attributed to unconfirmed LB (n=65) | 59 | 52 | 50 | 41 | 43 |
| | Population cohort (n=4000) | 4000 | 1544 | 1223 | 915 | 1212 |
| | Tick bite cohort (n=2405) | 2405 | 1584 | 1293 | 940 | 1111 |
| SF-36 (subscale bodily pain) | All LB patients (n=1135) | 1121 | 1017 | 968 | 887 | 924 |
| | *EM (n=1076)* | 1067 | 961 | 914 | 836 | 873 |
| | *Disseminated LB (n=59)* | 54 | 56 | 54 | 51 | 51 |
| | Chronic symptoms attributed to unconfirmed LB (n=65) | 59 | 52 | 50 | 41 | 43 |
| | Population cohort (n=4000) | 4000 | 1544 | 1223 | 915 | 1212 |
| | Tick bite cohort (n=2405) | 2405 | 1584 | 1293 | 959 | 1111 |

The numbers of missing questionnaires during follow-up was higher for the population and tick bite cohorts than for the LB patients, as the participants in these cohorts were not reminded by phone if questionnaires were not completed. For the population cohort, the follow-up questionnaires were optional.

Moreover, blood samples were collected from the cohort of LB patients (n=1044 out of 1135) at baseline and after six weeks, as well as from all 65 patients with chronic symptoms of unknown etiology attributed to LB, but without a confirmed LB diagnosis, at baseline.

[1]In the first year after start of inclusion, the CIS subscale fatigue was not included at the 3 and 9 months' time points. In the following years, the shortened form of the CIS was used at these time points.[4]

*Abbreviations: CFQ = Cognitive Failure Questionnaire, CIS = Checklist Individual Strength, EM = erythema migrans, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

## Supplementary Table S6: Statistical test results for comparison of prevalence of persistent symptoms between cohorts

| | Any symptom | | Fatigue | | Cognitive impairment | | Pain | |
|---|---|---|---|---|---|---|---|---|
| | *Population cohort* | *Tick bite cohort* | *Population cohort* | *Tick bite cohort* | *Population cohort* | *Tick bite cohort* | *Population cohort* | *Tick bite cohort* |
| **All LB patients** | <0.0001 | 0.016 | <0.0001 | 0.00089 | 0.0037 | 0.95 | 0.017 | 0.0091 |
| ***EM*** | 0.00012 | 0.021 | <0.0001 | 0.0014 | 0.0032 | 0.84 | 0.074 | 0.031 |
| ***Disseminated LB*** | 0.019 | 0.021 | 0.019 | 0.021 | 0.62 | 1.00 | 0.00040 | <0.0001 |

P-values belonging to Figure 2. The prevalence of persistent symptoms in LB patients (either all LB patients, or the group of patients with EM or disseminated LB) compared with the population and tick bite cohorts. Standardised prevalence for any symptom (according to the definition of persistent symptoms) and for each individual symptoms (fatigue, cognitive impairment and pain) are compared using permutation tests based on sum statistics.

*Abbreviations: EM = erythema migrans, LB = Lyme borreliosis.*

## Supplementary Table S7: Prevalence of persistent symptoms for the duration of three to six months

| | Reference cohorts | | LB patients | | |
|---|---|---|---|---|---|
| **Analysis** | **Population cohort** | **Tick bite cohort** | **All LB patients** | ***EM*** | ***Disseminated LB**** |
| Persistent symptoms for ≥3 and <6 months | 3.3% (2.4-4.2)<br>n=1942 | 3.5% (2.6-4.3)<br>n=1887 | 6.4% (4.9-7.8)<br>n=1084<br>[1] 0.00037<br>[2] 0.00026 | 6.2% (4.7-7.7)<br>n=1026<br>[1] 0.00072<br>[2] 0.00060 | 7.4% (0.9-14.0)<br>n=58<br>[1] 0.037<br>[2] 0.063 |

Results depict prevalence of persistent symptoms (95% CI) and total number of subjects with persistent symptoms for the duration of three or more months, but less than six months. Permutation tests based on sum statistics were used to assess differences in prevalence of persistent symptoms between LB patients (and EM and disseminated LB patients) and the population ([1]) and tick bite ([2]) cohort.

*Disseminated LB patients were stratified by two out of four confounders (comorbidity and sex).

*Abbreviations: CI = confidence interval, EM = erythema migrans, LB = Lyme borreliosis.*

## Supplementary Table S8: Statistical test results for comparison of symptom severity between cohorts

| Cohort | Reference cohort | Model parameter | p-value CIS | p-value CFQ | p-value SF-36 |
|---|---|---|---|---|---|
| *Comparison of mean standardised severity score over time (based on linear mixed effects model)* | | | | | |
| EM | Population cohort | EM | <0.0001 | <0.0001 | 0.0028 |
| | | Time point and EM | 0.0055 | 0.094 | 0.047 |
| | | Square time point and EM | 0.067 | 0.33 | 0.18 |
| | Tick bite cohort | EM | <0.0001 | <0.0001 | <0.0001 |
| | | Time point and EM | <0.0001 | 0.11 | 0.035 |
| | | Square time point and EM | <0.0001 | 0.20 | 0.090 |
| Disseminated LB | Population cohort | Disseminated LB | <0.0001 | 0.14 | <0.0001 |
| | | Time point and disseminated LB | <0.0001 | 0.022 | <0.0001 |
| | | Square time point and disseminated LB | 0.00027 | 0.17 | <0.0001 |
| | Tick bite cohort | Disseminated LB | <0.0001 | 0.11 | <0.0001 |
| | | Time point and disseminated LB | <0.0001 | 0.0026 | <0.0001 |
| | | Square time point and disseminated LB | <0.0001 | 0.058 | <0.0001 |
| *Comparison of mean standardised severity score at the 12 months' time point* | | | | | |
| EM | Population cohort | | <0.0001 | 0.022 | 0.00020 |
| | Tick bite cohort | | <0.0001 | 0.017 | 0.0040 |
| Disseminated LB | Population cohort | | 0.32 | 0.69 | 0.029 |
| | Tick bite cohort | | 0.19 | 0.88 | 0.0067 |
| *Comparison of mean standardised severity score over time (based on linear mixed effects model)* | | | | | |
| Chronic symptoms attributed to unconfirmed LB | LB patients with persistent symptoms | Group of patients with chronic symptoms attributed to unconfirmed LB | <0.0001 | 0.028 | 0.55 |
| | | Time point and the group of patients with chronic symptoms attributed to unconfirmed LB | <0.0001 | <0.0001 | <0.0001 |
| | | Square time point and the group of patients with chronic symptoms attributed to unconfirmed LB | 0.00016 | <0.0001 | 0.00050 |

P-values belonging to Figures S1 and S3. For the linear mixed effects models, p-values for the cohort fixed effects or interactions (the cohort, the interaction between cohort and time point, and the interaction between cohort and quadratic polynomial for the time point) are depicted. If any p-value is significant, the overall severity course of the cohort is considered to be different from the severity course of the reference course. For differences in severity scores between cohorts at 12 months, permutation tests based on sum statistics are used.

*Abbreviations: CFQ = Cognitive Failure Questionnaire, CIS = Checklist Individual Strength, EM = erythema migrans, LB = Lyme borreliosis, SF-36 = SF-36 item Health Survey.*

## Supplementary references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